

At page 2, please replace the paragraph beginning at line 22 with the following:

B¹ -- FIG. 5 is a graph showing the effect of ascorbic acid and glutathione on the number of TH-ir cells grown in a cell culture.--

At page 2, please replace the paragraph beginning at line 24 with the following:

B² --FIG. 6 is graphs showing the effects of ascorbic acid (AA), dopamine, and D-acetyl cysteine on the number of TH-ir cells grown in a cell culture.--

At page 8, please replace the paragraph beginning at line 4 with the following:

B³ -- As used herein, the term dopaminergic neuronal cells refers to those cells generally found in the region of the ventral midbrain (VM) known as the substantia nigra pars compacta that project to the striatum. The precursor cells are typically found near the midbrain/hindbrain junction of an intact brain. Dopaminergic neurons can be characterized by their secretion of dopamine as a neurotransmitter and high levels of expression of tyrosine hydroxylase (TH), an enzyme that catalyzes the rate limiting step in the biosynthesis of dopamine.--

At page 10, please replace the paragraph beginning at line 9 with the following:

B⁴ --Preferably, the differentiated media includes at least one of cAMP, forskolin, dopamine and ascorbic acid. Preferably, cAMP is present in a concentration from about 1 μ M to about 5 mM, more preferably about 10 μ M to about 1 Mm. Typically, differentiation media containing cAMP results in about a 50% to about 300% increase in the total number of differentiated neural cells from precursor cells. Most typically, an increase in tyrosine hydroxylase immunoreactive (TH-ir) cells, also called dopaminergic cells is observed. Preferably forskolin is present in the differentiation medium at a concentration from about 1 μ M to about 100 μ M, more preferably about 2 μ M to about 10 μ M. Typically, differentiation media containing forskolin results in about a 40% to about 150% increase in the total number of differentiated neural cells, particularly TH-ir cells. Preferably dopamine is present at a concentration from about 0.1 μ M to about 1 mM, more preferably about 1 μ M to about 1 mM to provide about a 300% to about 700% increase in the total number of TH-ir cells. The effects of cAMP, forskolin and dopamine appear to be additive.--

At page 16, please replace the paragraph beginning at line 17 with the following:

B5
--Tissue pieces were spun in a centrifuge at 209g for 5 minutes and mechanically triturated to a quasi single cell suspension in HBSS and counted. 5 ml of cell suspension at a concentration of $150 - 200 \times 10^3$ cells/ml was plated on a 10 cm culture dish containing DMEM/F12/N2 medium (Bottenstein, J.E. & Sato, G.H. Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc.Natl.Acad.Sci.USA 76, 514-517 (1979) (modified according to Johe, K.K., Hazel, T.G., Müller, T., Dugich-Djordjevic, M.M. & McKay, R.D.G. Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. Genes and Development 10, 3129-3140 (1996)) that had been previously coated with polyornithine (15ug/ml) and fibronectin (1ug/ml).--

At page 19 please replace the paragraph beginning at line 5 with the following:

B6
--Using general immunohistochemical procedures known to those of skill in the art, the immunoreactivity of the cells to TH was examined using TH polyclonal 1:500 (Pel Freeze). While no tyrosine hydroxylase (a rate limiting enzyme in the synthesis of dopamine) immunoreactive (TH-ir) cells could be detected in the tissue at the time of dissection, after 7 days of differentiation 18.4% +/- 5.1% of the total cell population was immunoreactive for TH. TH-ir cells were also immunoreactive for dopamine, dopamine transporter (DAT) and for TuJ1. The percentage of TH-ir cells in non-expanded cultures, grown without bFGF was 5.6 +/- 1.3%.--

At page 22, please replace the paragraph beginning at line 19 with the following:

B7
-- Lipid peroxydation is a colorimetric assay (OXIS BIOXYTECH® LPO-568™; Oxis International Inc.) and was used according to the specifications of the manufacturer. H_2O_2 assay measures the conversion of 2',7' dichlorofluorescein diacetate (Molecular Probes d-399) into 2',7' dichlorofluorescein catalyzed by H_2O_2 . The assay was performed according to the specifications of the manufacturer. Immunohistochemistry for oxidative stress markers by the DAB-peroxidase technique was performed on cells differentiated in the presence or absence of ascorbic acid, glutathione or D-acetyl-cysteine plus ebselin. The following antibodies were used:

B7
cont.

Epitope	Antibody Type	Catalogue No.	Company
Anti-hemoxygenase I	monoclonal AB	OSA-111	Stressgen
Anti-8-hydroxyguanosine	monoclonal AB	12501	QED Bioscience Inc.
Anti-Nitrotyrosine	polyclonal (sheep) AB	24312	Oxis International Inc.--

At page 23, please replace the heading at line 28 with the following:

--E. **Combination treatment with cAMP, ascorbic acid and dopamine--**

At page 23, please replace the paragraph beginning at line 29 with the following:

B8

--Combination treatment experiments were carried out with mesencephalic precursors expanded with bFGF for 11 days and differentiated in DMEM/F12/N2 medium in the presence or absence of ascorbic acid (100uM), dopamine (1nM-1mM) and cAMP (1mM). The data revealed that dopamine has only a very minor additional effect on the yield of dopaminergic neurons as compared to ascorbic acid treatment alone (see Figure 6). cAMP and ascorbic acid have an additive effect on the yield of dopaminergic neurons from expanded precursors. Combination treatment of ascorbic acid and dopamine and of ascorbic acid and cAMP showed that dopamine contributes no significant additional effect to ascorbic acid treatment. The effects of ascorbic acid and cAMP appear additive.--

At pages 24-25, please replace the paragraph beginning at page 24, line 32 with the following:

B9

-- The differentiation medium for reaggregate cultures consisted of Neurobasal[®]/2%B27[®] (Gibco, Life Technologies) with or without fetal bovine serum (FBS; 10%, Gibco), glial derived neurotrophic factor (GDNF; 10ng/ml; Peprtech), brain derived neurotrophic factor (BDNF; 10ng/ml; Peprtech), neurotrophin 4/5 (NT4/5; 10ng/ml; Peprtech), SHH (2.5µg/ml; kindly provided by Dr. Thomas Muller, at the Labrotory of Molecular Biology, currently at the Max Delbrueck Univ. in Berlin; SHH is now commercially avail. fro R&D).--